

Characterization of Nubian and Nilotic Goats By Random Amplified Polymorphic DNA Technique

By: *Hiba Abbas Ibrahim Mohamamd Ahmed*

**B.Sc. Department of Chemistry Faculty of Education
University of Khartoum**

**A thesis Submitted to the University of Khartoum in Partial for Fulfillment for the
Requirements for the Degree of M.Sc. In Biochemistry**

Supervisor: *Dr. Osman Mohamed Elsheikh*

Associate Prof. Biochemistry Ph.D. M.Sc. B.Sc.

**Department of Biochemistry - Faculty of Veterinary Medicine
University of Khartoum**

September 2007

g

الآية

قال تعالى:

«ولهم فيها منافع

ومشارب أفلا

يشكرون»

صدق الله العظيم

السورة : يس

الآية : (73)

List of Contents

subject	No .page
List of Contents	i
List of Tables	iii
List of Figures	iv
Dedication	v
Acknowledgment	vi
English Abstract	vii
Arabic Abstract	viii
Introduction and literature review	1
Chapter one	
Literature review	3
1.1. Goat in Sudan	3
1.1.1. Nilotic goat	3
1.1.2. Mountain goat	4
1.1.3. Desert goat	5
1.1.4. Sudanese Nubian goat	5
1.2. Polymerase Chain Reaction (PCR)	6
1.3. DNA markers	7
1.3.1. Restricted Fragment Length Polymorphism (RFLP)	8
1.3.2. Amplified Fragment Length Polymorphism (AFLP)	9
1.3.3. Microsatellites	10
1.4. Application of genetic markers	11
1.4.1. Genome mapping	11
1.4.2. Quantitative trait loci	12

1.4.3. Biodiversity study	12
1.5. Random Amplified Polymorphic DNA	13
1.5.1. Studies that uses RAPDs	14
1.5.2. The advantages and disadvantages of RAPD	15
Chapter Two	
Materials and Methods	17
2.1. Materials	17
2.1.1. Animals	17
2.1.2. Samples Collections	17
2.2. Isolation of Genomic DNA	17
2.2.1. Extraction and purification of genomic DNA	18
2.2.2. DNA electrophoresis	20
2.3. PCR protocol	21
2.3.1 Preparation and optimation of PCR	21
2.3.2. Electrophoresis of PCR products	22
2.3.3. Thermal cycling program	25
2.4. Statistical analysis	26
Chapter Three	
Result	27
3.1. Genotypic characteristics	27
3.2. PCR product of primer Q19	29
3.3. PCR product of primer K16	29
3.4 PCR product of primer G12	29
3.5. PCR product of primer Q3	29
3.6. PCR product of primer K3	30

Chapter Four	
Discussion	36
Conclusion	38
Recommendation	39
References	40
Appendix 1: Nubian goat	48
Appendix 2: Niltoic goat	49
Appendix 3: Ladder molecular size of DNA	50

List of Tables

Subject	No .page
Table (1): Sequence of Oligonucleotide primer used in the random genes.	23
Table (2): PCR component.	24
Table (3): statistical result for the RAPD techniques by primer Q19.	31
Table (4): statistical result for the RAPD techniques by primer K16.	32
Table (5): statistical result for the RAPD techniques by primer G12.	33
Table (6): statistical result for the RAPD techniques by primer Q3.	34
Table (7): statistical result for the RAPD techniques by primer K3.	35

List of Figures

Subject	No .page
Figure (1): PCR product for primer K16.	25
Figure (2): PCR product for primer Q19.	27
Figure (3): Frequency of primer Q19.	28
Figure (4): Frequency of primer K16.	32
Figure (5): Frequency of primer G12.	34
Figure (6): Frequency of primer Q3.	34
Figure (7): Frequency of primer K3.	35

Dedication

To my father ...My mother

My brothers

My teachers ...My

husband

Acknowledgment

I would like to thank Dr. Osman Moahmmed Elsheikh. Without his help this research wouldn't have been a reality. I sincerely appreciate the technical help of Dr. Galal Moahmmed Yousif. They were very generous to see me at their office, at any time and they did not spare any chance to help me with advice, materials and references.

I also like to give my gratitude to the staff in University of Khartoum, Faculty of Veterinary Biochem dept. for the chance

they afforded me to enjoy such a nice program .

My deep heart appreciation extended to my colleagues in Khartoum University -Faculty of Education who kept asking about my progress in my master's research.

Abstract

This is a characterization study to differentiate between the Nubian and Nilotic goats breeds. Vein blood samples (20 each) were collected from the animal research center at Hillat Koko, Khartoum North, Sudan. 2007

Eight random primers were tested using random amplified polymorphic DNA technique (RAPD). These primers gave PCR products for Chinese goat.

Results revealed that the primers Q19, K16, G12, Q3 and K3 showed a clear and reproducible PCR products for 85%, 100%, 95%, 47.5% and 67.5% of the studied goats. Frequency of distribution of these primers ranged from 0.4 to 1.0. the number of nitrogen bases were in the range of 200-700 base pairs.

Although the studied population was small but the data obtained in this study indicated great similarities between the Nubian and Nilotic goats with respect to the studied primers.

(20)

.

2007

(RAPD) DNA

.(PCR)

K3 Q3 G12 K16 Q19 .

%67.5 %47.5 %95 %100 %85

1.0-0.4

.

.700-200

.

Introduction

Sudan occupies geographical position in the north eastern quarter of the African continent with access to eight African countries and the open boundaries of the Sudan make the migration and travels of animals from Sudan to these countries and vice versa easy. This may lead crossed then to be with breeds of these countries, and lead to the loss of native genetic potential.

The Ministry of Animal Resources (2005) reported that the goat population in Sudan about 41.5 million which represent a very large population compared to other African countries. The estimates of FAO (1999) indicated that there were 37.5 million head of goats in Sudan. Therefore, goats in Sudan are increasing and play high important role in the economy of the country. Goat contributes to the health and nutrition of several million people in developing countries, especially those on the poverty line. Rearing goats provides a small but important supply of animal's proteins of high biological value, plus essential minerals and vitamins which are of particular significance for the most vulnerable group namely pregnant and nursing mothers and young children (Devendra and Burns, 1983). Small number of foreign breeds of goats was imported into Sudan mainly Damascus, Anglo-Nubian, Saanen and Toggenburg goats.

With the advancing of DNA chemistry and molecular genetic, it has been possible to improve genetic potential of plants and animals. Analysis of the DNA and genes can provide basic information that may

be used for introduction of excellent productive traits to the local animals. In fact all previous traits in breeding programs depend on the studying of morphological characteristics (phenotype), which reflects the genotypic structure of creatures.

In this study a molecular marker technique (RAPD) was used for studying the characterization of the Nilotic and Nubian goats which are local breeds in Sudan. It is based on a modified PCR method by using short oligonucleotide primers. RAPD analysis has found many uses in diverse fields such as the assessment of genetic diversity among investigated species or classification of taxa in both plants and animals (Borowsky et al., 1995).

Objectives of this study

- 1- To use molecular biology technique (RAPD) to identify Nubian and Nilotic goats
- 2- To obtain better description and knowledge of genetic resources in the Nubian and Nilotic goats population

Chapter One

Literature Review

1.1. Goats in Sudan

Goats in Sudan were estimated as 41.5 million heads (animal's resources statistics, 2005). This large population is mainly comprised of many unspecified ecotypes widely distributed in all ecological zones from arid northern regions to humid southern Sudan. According to AOAD (1990), four distinct and well known types can be enumerated as Nilotic, Mountain, Desert and Sudanese Nubian goats.

1.1.1. Nilotic goat

It is known as dwarf, equatorial or southern Sudan goat. It is a dwarf type characterized by variable physical features of compact body and smaller in size only half the size of other Sudanese goats. A live weight range from 11-25 kg (Mason, 1951; Elnaim, 1979; AOAD, 1990) the forehead is convex and facial profile is straight. The ears are of medium size. Both sexes usually carried small horns. The male is bearded and maned.

The color is variable and nearly all colors are found but the most common is mixture of black and whitish hairs (Mason, 1951; Elnaim, 1979; AOAD, 1990).

Nilotic goats are distributed throughout the southern Sudan. South to the 11th parallel latitude. They are well known to be trypanosomiasis

resistant. Three varieties of the Nilotic goat can be distinguished: Toposa (Mangala) goat which are mainly found in Eastern equatorial state and characterized by comparatively large size, Yei (Dinka) goat which are located in western equatorial and latuka Bari goat in Jongelli state (Mason, 1951, AOAD, 1990; Muffarrah, 1995).

1.1.2. Mountain goat

Known also as Tiegri goat. It is a small dwarf goat characterized by very short ears carried horizontally, short neck and legs, with live weight ranges 20-30 kg, and both sexes are horned. The horn on the male is longer, straight and slightly backward elevated. The male is beard and mane. Sometimes wattles are present in both male and female. The coat is short with black lines extended along the face from the base of the horns to the nostrils. The most common color is dark brown or grey brown (Elnaim, 1979; Muffarrah, 1995). However, Mason (1951) classified it as a variety of the Nilotic goat.

The mountain goat varieties are Nuba, Toker, and Ingessena goats. They are distributed in Nuba Mountains (southern kordofan state), Ingessena mountains (Blue Nile state) and red sea mountains (red sea state) at Toker and Haliab districts (AOAD, 1990).

1.1.3. Desert goat

This is hardly type of goat, well adapted to arid, semi-arid and savannah regions of western Sudan. It is characterized by wide variability in physical characters and variable size ranging from medium to large size. Ears are usually shorter than Nubian goat and protrude to the outside. Both sexes carry twisted horns commonly projected outwards. The beard of male is well developed. The coat is short, of different colors but mainly grays and often splashed with brown or black. All males and females carry a diffused bushy mane which extends on the anterior part of the neck (Mason, 1951; Elnaim, 1979; Kiwuwa, 1986; AOAD, 1990).

The desert goat is mainly found in western Sudan in kordofan and Darfour states, also cross-breed types between Desert and Nubian are found in White Nile and Blue Nile states (Elnaim, 1979; AOAD 1990).

1.1.4. Sudanese Nubian Goat

The breed was developed along the river valley of southern Egypt (wawat) and northern Sudan (Kush).

The synonym is derived from the Nubian tribe (kiwuwa, 1986). It is dairy type goat characterized by fairly proportioned body size with small to medium size head, convex facial profile and large drooping ears usually turned out of the lower tips. Both sexes carry medium sized lateral or backward sweeping horns which are simple in females but slightly twisted in males, some are polled goats especially females. The

neck is of moderate length, the chest is deep and the withers are prominent. The back is long and straight. The legs are long, stronger and well proportioned. The udder is large and well shaped (Mason, 1951; Elnaim 1979; AOAD, 1990; Kiwuwa, 1986).

The coat is relatively large. Rudimentary mane, beard and wattle exist in the male. The color is commonly black (Mason, 1951) but pure brown, pure white and different shadows between them are found. Also multi-colorations of black and white are found (Elnaim, 1979; AOAD, 1990).

Nubian goat distributed in river in Sudan north to the 12th latitude (Mason, 1951). The Sudanese Nubian goat belong to the general Nubian group which characterized by the roman nose, large loop ears, relatively short silky hair of various colors and scimitar shaped back ground sweeping horns (Mackenzie, 1951; French, 1970). In Africa, the Sudanese Nubian resembles the zaraibi type goat in Egypt and the shokeria goat in Eritrea (Mason, 1951).

1.2.Polymerase Chain Reaction(PCR)

The polymerase chain reaction (PCR) is a powerful technique for highly specific amplification of DNA defined by two flanking primers and has had a major impact on many aspects of biology (Mullis et al 1987). This allows the amplification or reproduction in great amount of particular regions of the DNA. In order to initiate the process of replication of the DNA. A reproduction or amplification of thousands of copies of a chromosomal region or gene of interest is obtained by

repeated cycles of synthesis and denaturalization (chain separation) of the DNA using temperature changes. Since the primers are specific technique of DNA corresponding to thousand of genes are arranged on small matrices and probed with labeled cDNA from a tissue of choice. The information is then read by a device to be down loaded to a computer (Schimenti, 1998).

1.3. DNA MARKERS

Genetic markers associated with DNA are commonly grouped into type 1 and type 2 markers (O'Brien, 1991). Type 1 markers are usually associated with a gene with known function, while type 2 markers refer to anonymous gene segments. Alternatively, DNA-based markers are also grouped as clone/sequence-based markers are also grouped as markers (CSB) such as Microsatellites and fingerprint fragment and the determination of the sequence of the fragment. Some fingerprint markers rely on random recognition sites e.g. (RAPD: randomly amplified polymorphic DNA) and thus don't require the availability of specific DNA sequence information (Dodgson et al., 1997).

The specific genetic marker techniques:

1.3.1. Restricted fragment length polymorphism (RFLP)

This technique relies on the amplification of variable regions of the target genome, with the amplicon then being digested with one or more sequence specific restriction enzymes. The DNA fragments of different lengths are then subjected to electrophoresis and fragments will migrate according to their weights, the smaller fragments faster and the larger fragments slower (Nicholas, 1996). Thus, RFLP generally refers to the difference in banding patterns obtained, from DNA fragments, after sequence specific cleavage with restriction enzymes. Before the advent of PCR, RFLP analyses typically entailed RE digestion of chromosomal DNA, followed by electrophoresis separation, membrane blotting and hybridization with a labeled probe, usually radioactive. Although reliable, this process is time-consuming, cumbersome and expensive. However, the ability to amplify a selected target million fold through PCR abolished the need for the problematic blotting and hybridization analyses. Although many enzymes still need to be tested in the initial phase to be able to identify the polymorphism, it is today an easy, reliable and relatively cheap marker to use (Dodgson et al., 1997) in comparisons of sequence polymorphism. Restriction enzymes have often been shown (BamHI, EcoRI SacI and Taq) to be useful in obtaining RFLP patterns for haplotype identification in individuals (Spike et al., 1996; Smith et al., 1996). Potential disadvantages of the RFLP technique are the dimorphic nature, since an

RFLP only indicates the presence or absence of a cleavage site, and therefore doesn't provide a great deal of genotypic information.

1.3.2. Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting technique that is based on the detection of DNA fragments, subjected to restriction enzymes, followed by selective PCR amplification (Matthes et al., 1998; Karp et al., 1997). AFLP procedures can be manipulated to suit specific applications through selection of the restriction enzymes and the design of the PCR primers. Typically a rare-cutter and frequent-cutter restriction enzymes is combined to ensure the generation of small fragments and RFLP is therefore probably the simplest PCR procedure for the same time. PCR primers can be designed to have no selective bases on the 3' end if the targeted templates are simple elements such as plasmids or bacterial artificial chromosomes. Like in other techniques for fingerprinting, fragments are separated and analyzed using gel electrophoresis. The AFLP technique can be performed at reasonable cost, has the advantage of a higher responsibility than RAPD, and has been used extensively in plant genome mapping (Vos et al., 1995). The major advantage of the AFLP technique is the large number of polymorphisms that the method generates. Its ability to differentiate individual in population makes the technique useful for paternity analyses (Krauss, 1999). More recently this technique has also begun to find application in genetic studies of livestock with successes reported for example in cattle and chickens

(Nijman et al., 1999; Herbergs et al., 1999). Nakajima et al., (1998) found that AFLP methods produced on average four times as many bands per reaction compared to RAPDs in their analysis of *Daucus* (apiaceae) diversity.

1.3.3. Microsatellites

Microsatellites alternatively known as simple sequence repeats (SSRs), short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs) are tandem repeats of sequence units generally less than 5 Pb in length (Bruford and Wayne, 1993; Tautz & Renz, 1984; Tautz, 1989). Microsatellites are highly polymorphic due to the variation in the number of repeats. It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small number of samples (Goldstein and Polack, 1997).

A large number of Microsatellites markers have been mapped for various species, including humans, mice, fruit flies and farm animals (cattle, sheep, swine and chickens)(Goldstein and Pollack,1997; Groenen et al., 2000).

The great advantage of Microsatellites analysis is the large number of polymorphism that the method reveals, as with AFLPs (cregan et al., 1995).

The problem with use of Microsatellites is mutation in the binding region of one or both Microsatellites primers or by inhibit annealing that may result in the reduction or loss PCR produced (callen et al., 1993). These null alleles may be manifested as fewer heterozygote than

expected in randomly mating population or by the appearance of “empty” lanes (Morgante et al., 1998).

1.4. Application of genetic markers

A very exciting and fast developing application of genetic markers is in the mapping of various animal genomes. Conservationists also use various genetic markers in evolutionary and genetic biodiversity studies.

1.4.1. Genome mapping

Livestock genome mapping is a complex and time consuming effort, but once completed holds promise for finding functional genes, quantitative trait loci (QTL's) and genes associated with disease resistance. The first genetic linkage map was constructed for chickens (Bumstead and Palyga, 1992), followed by mapping of cattle, pig and sheep genome.

Further important aspects of genome mapping are the comparison of maps of different species. Comparative mapping is in process between humans and various farm animal species (Dodgson and Cheng, 1999; Hayes et al., 2002).

Comparative mapping has several potential advantages, firstly identification of conserved regions between species; secondly it contributes to the search QTLS and also provides valuable information for gene expression studies.

1.4.2. Quantitative Trait Loci (QTL)

Quantitative trait loci (QTL) are referred to as loci affecting quantitative traits. In farm animals most of the performance observed in traits of economic importance are the result of quantitative variation, which emphasizes the interest in finding these QTL. In order to identify a QTL for specific trait, many animals have to be genotyped for a large number of markers on different chromosomes. Phenotypic data for the trait are also required. By means of statistical analysis genetic and phenotypic data are combined and it is possible to find the most likely location on the chromosome responsible for the specific trait. (Walsh, 2001).

There are number of mapping projects underway searching for QTL in pigs. Traits affecting reproduction such as age at puberty, ovulation rate and testicular size, and growth and carcass traits are included in the various projects (Rohrer and Keele, 1998a & 1998b; Wang et al., 1998).

1.4.3. Biodiversity studies

It is inevitable that selection, inbreeding and various crossbreeding systems may lead to the loss of genetic variation within breeds and that the breed itself may become extinct. For this reason, the scientific community identified the need for conservation of livestock resources. During 1992 the food and agricultural organization (FAO) launched a program for global management of farm animal genetic resources, with the main objective being to stimulate conservation activities and create an awareness of possible losses of genetic

resources on an international basis. A program was launched for all the different farm species requiring genetic characterization with DNA markers. Genetic markers such as DFP`S, RAPD and Microsatellite have been used in studying genetic variability in cattle, sheep, goats, chickens, pigs, swine and horses (MacHugh et al., 1998; Buchanan et al., 1994; Vanhala et al., 1998; Van Zeven et al., 1995; Kruger et al., 2002).

1.5. Random Amplified Polymorphic DNA (RAPD)

In 1990 Williams et al. Reported anew method for identifying genetic polymorphisms called the random amplified polymorphic DNA (RAPD) assay. This is the simplest of all PCR based markers. Random pieces of the genome of 300 to 2000 base pairs (bp) long are amplified using a single oligonucleotide primer of arbitrary sequence to amplify anonymous fragment of genetic DNA (Welsh and McClelland 1990). The technique requires no prior knowledge of DNA sequence to generate genetic markers and is fairly simple (Bowditch et al., 1993). A discrete PCR product is produced when the primer binds to sites on opposite strands of the DNA that are within an amplified distance (less than 3000 bp usually). The presence or absence of this specific product is thought to represent mutation in the primer-binding sites of the DNA (Clark, Lanigan 1993). RAPD reactions normally amplify three to ten different pieces of DNA.

Primers used are about 10 bp long and because of this short length, the annealing temperature must be low (35-40C). Products are realized on

agarose gel and stained with ethidium bromide (polyacrylamide gel and silver staining can also be used). There are variations on these techniques, using shorter primers (5 bp) or two arbitrary primers. As well as alternative methods of staining and fragment resolution.

Two modifications of RPAD markers have been described as DNA Amplification fingerprinting (DAF) and Arbitrary Primed PCR (AP-PCR). DAF uses short random primers of 5-8 bp and visualize the relatively greater number of amplification products by polyacrylamide gel electrophoresis (PAGE) and silver staining. AP-PCR uses slightly longer primers and amplification products are radiolabelled and also resolved by PAGE.

1.5.1. Studies That Uses RAPDs

At present, research on goats is woefully inadequate compared to that on sheep and swine, even though a number of research projects have been conducted on goats over the past 20 years. There are even fewer studies on goat genetics using molecular methods (Morand-Fehr, 2004). As result, the genetic background of some goat populations remains largely unknown.

Since its inversion by Williams et al. (1990). The randomly amplified polymorphic DNA (RAPD) technique has been used to develop molecular markers to study the genetic diversity among species, as well as to examine the genetic variation and relationship within populations. (kanntanen et al., 1995;Cushwa et al., 1996;Smith et al., 1996;

Mollenbeck, 1999; Qian and Ge, 2001; Larrasa et al.,2000; Singh RV,Sharma, 2002; Yu et al., 2004).

Lee and Chang, (1994) utilized a single RAPD primer to generate species–specific fingerprinting that permitted distinction between nine different species (bovine, chicken, dog, duck, goat, human, pig, rabbit, and rat).The authors concluded that the RAPD assay provided a relatively simple, fast, and sensitive species-identification fingerprinting method that would be useful in analyzing crime scene evidence and identifying products from endangered species.

Cargill et al. (1995) utilized the pooled DNA screening approach to identify a sheep–versus goat-specific RAPD markers.

1.5.2. The advantages and disadvantages of RAPD

Each genetic marker technique has both advantages and disadvantages, which is the impetus of occurrence and development of molecular genetic marker method.

The one of the advantages of the RAPD assay is that the products can be separated on agarose gels, which are typically easier to manipulate than non denaturing polyacrylamide gels. However, the decreased resolution of agarose gels can potentially result in scoring co-migrating fragments of similar size as homologous (Hardrys et al., 1992; Amau et al., 1994).

One of shortcoming of RAPD is its low and repeatability and occurrence of pseudo-bands. The shortcoming could be improved through controlling suitable condition of amplification reaction, that is

preventing DNA extracted from contamination of external impurities and keeping amplification condition stable (Haberfeld et al., 1991; Welsh et al., 1990).

Chapter Two

Material and Method

2.1. Material:

Material used in the study are animal for collection of genetic material (DNA), Primers and reagents necessary for PCR.

2.1.1. Animal

A total of 40 animal representing 2 local breeds of goat (Nubian, Nilototic) were selected from animal production research center .of Hilat Kooko /Khartoum North , Sudan .

2.1.2. Samples Collection :

Under aseptic condition 40 blood samples (20 Nubian ,and 20 Nilototic) were taken from jugular vein of each, using sterile syringes transferred to cationers containing EDTA as anticoagulant, and kept at 4C.

2.2. Isolation of genomic DNA

Blood samples were utilized for extraction and purification of the gentic DNA of Nubian and Nilotic goads .

2.2.1. Extraction and purification of genomic DNA

Genomic DNA was isolated and purified according to Promega Corporation (2005) by Wizard genomic DNA purification kit. The materials needed for the processing in addition to the DNA purification kit are:

- Sterile 1.5 ml microcentrifuge tubes
- Water bath 37 C
- Isopropanol, at room temperature
- 70% ethanol at room temperature
- Centrifuge
- Vortex mixer

The steps of extraction and purification were as follows :

1. 900 µl of cell lysis solution were added to a sterile 1.5 ml microcentrifuge tube.
2. The tube of blood was gently rocked until thoroughly mixed; then the blood was transferred to the tube containing the cell lysis solution. The tube was inverted 5-6 times to mix.
3. The mixture was incubated for 10 minutes at room temperature (inverted 2-3 times once during incubation) to lyse blood cells. The sample was centrifuged at 13000-16000 xg for 20 seconds at room temperature.

4. The supernatant was removed and discarded as much as possible without disturbing the visible white pellet, and Approximately 10-20 μ l of residual liquid will remained in the 1.5 ml tube.
5. The tube was vortexed vigorously until the white blood cells were resuspended (10-15seconds).
6. 300 μ l of nuclei lysis solution were added to the tube containing the resuspended cells. The solution was pipetted 5-6 times to lyse the white blood cells until the solution become very viscous. After mixing the solution was incubated at 37 C until the clumps were disrupted.
7. 1.5 μ l of RNase solution was added to the lyaste and mixed by inverting the tube 2-5 times. The mixture was incubated at 37 C for 15 minutes, and then cooled to room temperature.
8. 100 μ l of protein precipitation solution were added to the nuclear lyaste and vortexed vigorously for 10- 20 seconds. Small protein clumps were seen after vortexing.
9. The samples were centrifuged at 13000-16000 xg for 3 minutes at room temperature. A dark brown protein pellet was seen.
10. The supernatant was transferred to a clean 1.5 ml micro centrifuge tube containing 300 μ l of isopropanol. (at room temperature).

11. The solution was gently mixed by inversion until white thread-like strands of DNA form visible mass.
12. The samples were centrifuged at 13000-16000 xg for 1 minute at room temperature. The DNA was seen as a small white pellet.
13. The supernatant was decanted and one sample volume of (room temperature) 70% ethanol was added to the DNA. The tube was gently inverted several times to wash the DNA pellet and the side of microcentrifuge tube, and then centrifuge as in step 12.
14. Ethanol was carefully aspirated by a drawn pasture pipette. The tube was inverted on clean absorbent paper, and the pellet was air dried for 10-15 minutes.
15. 100 µl of DNA rehydration solution were added to the tube and the DNA was rehydrated by incubating at 2-8 C.

2.2.2. DNA electrophoresis:

The extracted samples were analyzed by electrophoresis to confirm the presence of DNA before doing amplification by PCR.

The preparation and examination of a gel were as follows:

- 0.8% agarose solution was prepared in the TBE buffer.
- The TBE buffer (10XTBE) molecular-biology grade which holds a PH of (8.3±0.1).
- The solution was prepared by heating in a water bath.

- The agarose solution was cooled to 50°C by air – dry for 5 minutes.
- 0.5 µl of Ethidium bromide was added to the solution.
- The solution was poured in to the gel plate.
- The comb was removed, after the solution gelled.
- The TBE (1xTBE) electrophoresis buffer was added to apparatus to a volume of about 3 ml above the gel.
- 1 µl of loading dye plus 5 µl of DNA were placed in the sample wells.

2.3 PCR protocol:

primers table (1) of NTPs, Tag DNA polymerase, PCR reaction buffer and MgCl₂, were obtained from promegaCo. USA. The procedural steps of PCR were performed as described by the authors of primers (Lili, 2 up 6) designed with minor modifications.

2.31. Preparation and optimization of PCR:

To obtain PCR product of the extracted DNA, reactions conditions were optimized by adjusting reaction mixture and thermal cycling program.

2.3.2. PCR reaction mixture:

The PCR reaction mixture contains 5X green Go tag reaction buffer (2.5 µl), 500U go tag DNA, polymerase (0.2 µl) 10 mM d NTPs mix (5 µl), 25 mM Mg Cl₂ (2 µl), 10 µM each primer (5 µl), template DNA (2 µl) and double distilled water (3.3 ML) to make the final volume 20 µl (Table2).

2.3.3. Electrophoresis of PCR products

Amplified samples were analyzed by electrophoresis separation in 1.5% agarose gel, 5 µl of each PCR product was mixed with 1 µl of loading dye and loaded into the wells of the gel. The gel was run at 100 volts for 1 hour.

After electrophoresis the gel PCR products were visualized with a UV transilluminator. Gene sizes were scored by using DNA marker 100 bp.

Table (1): Sequences of primers used.

PRIMER	SEQUENCE (5-3)
A11	CAATCGCCGT
A16	AGCCAGCGAA
G12	ACGCACAACC
K3	CCAGCTTAGG
Q19	ACAACGCCTC
Q3	CCGCGTCTTG
Q7	CCCCGATGGT
K16	AAGTCCGCTC

Primers were obtained from promega -Co USA were described by Lili et.al,2006

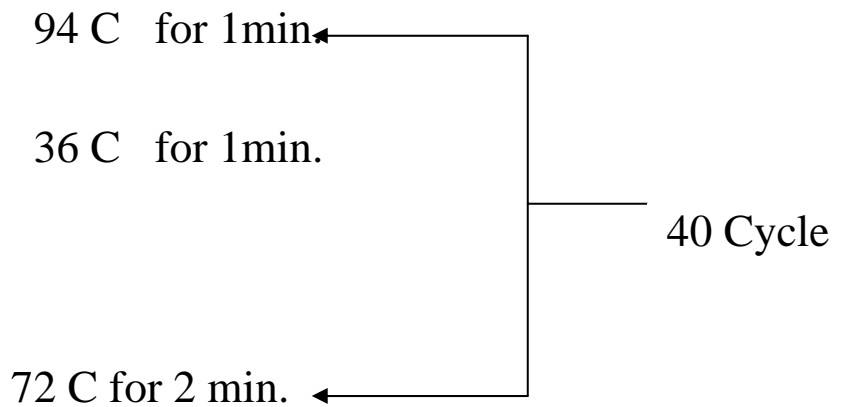
Table (2): PCR component

Component	Amount of one PCR reaction
d.d H ₂ O	3.3μl
10 x reaction buffer	2.5μl
Mg ₂ Cl	2.0μl
dNTPs	5.0μl
Primer	5.0μl
Taq polymerase	0.2μl
Template DNA	2.0μl
Total volume	20μl

2.3.4. Thermal cycling program:-

Amplification of DNA was carried out in a thermocycler programmed for 40 cycles as follows:

-Pre- denaturing 94 C for 2 min



-Final Extension 72 C for 10 min.

Then followed by soaking at 4 C.

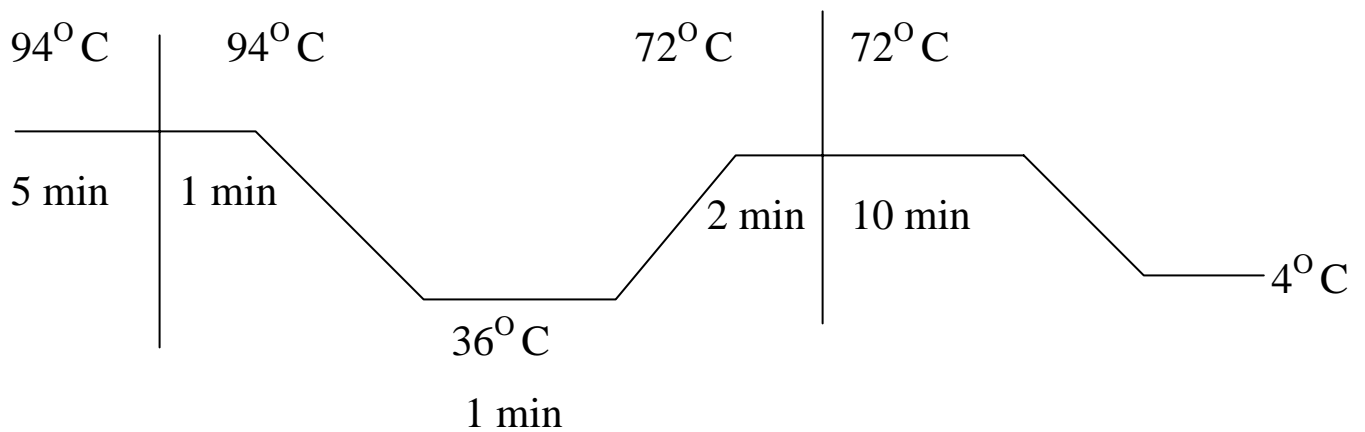


Fig.(1) Diagram of PCR operation

2.3.5. Statistical analysis:

Data obtained were processed, using Excel program for windows Microsoft. Frequencies, percentage and graphs were used for presentation of the data.

Chapter Three

Results

Genotypic characteristics

In this pilot study some genotypic characnizations of two local goads breeds were investigate using eight random primers. Results indicated that fine of the tested primers (G12, K3, Q19, Q3 and the other (A11, A16 and Q7) were negative.

1	2	3	4	5	6	7	8	9	10	11
---	---	---	---	---	---	---	---	---	----	----

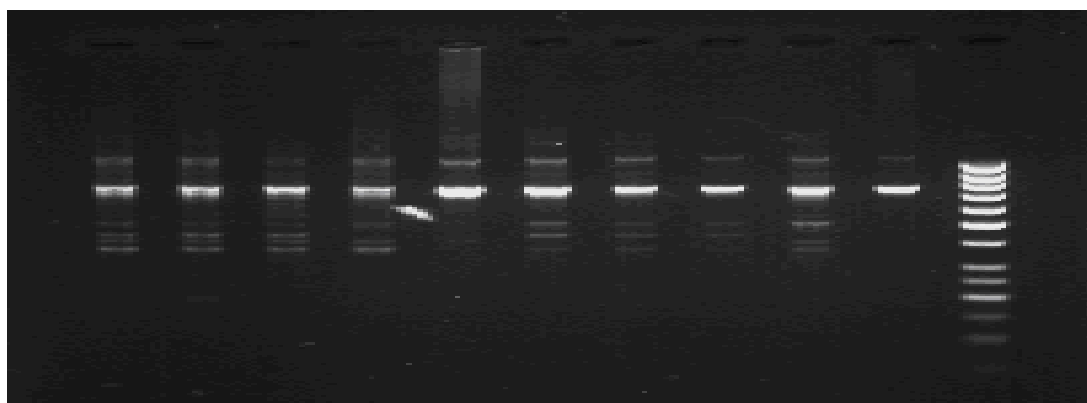


Fig (2): Electrophoretic pattern, shows PCR product (1.6% agrose gel) for primer K16 for Nubian and Nilotic goats.

Lanes, 1,2,3,4 and 5 Nilotic goats, lanes 6,7,8,9 and 10 Nubian goats lane 11 molecular size of DNA marker (100 bp).

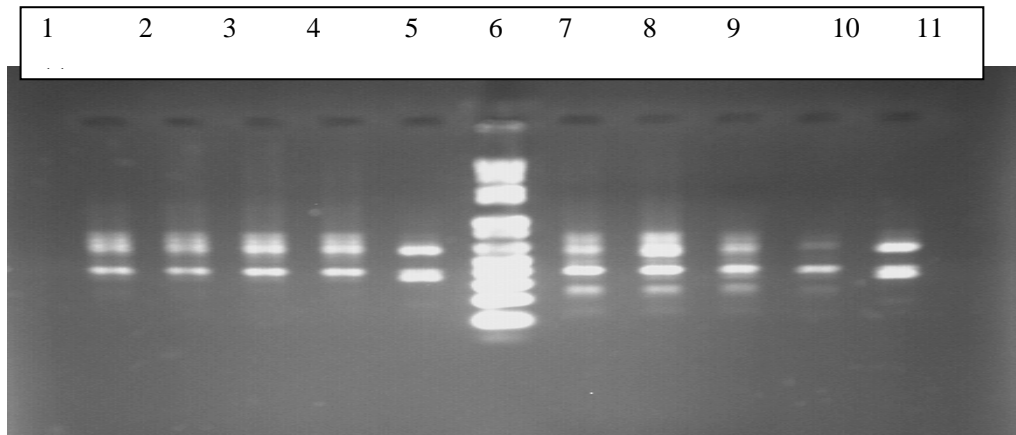


Fig (3): Electrophoretic pattern, shows PCR product (1.6% agarose gel) for primer Q19 for Nubian and Nilotic goats. Lanes, 1,2,3,4 and 5 Nilotic goats, lanes 7,8,9,10 and 11 Nubian goats lane 6 molecular size of DNA marker (100 bp).

PCR products of Q19 primer:

Data presented in table (3) indicated that about 85% of the local breeds gave PCR product for Q19 primer. The Nilotic goats gave slightly higher PCR product (45%) relative to the Nubian (40%). The calculated frequency of the PCR product of Q19 primer is 0.8 and 0.9 for the Nubian and Nilotic goats respectively, (Fig. 3). The number of nitrogen bases (base pairs) ranged from 200 to 700 bp.

PCR products of K16 primer:

Results presented in table (4) indicated the Nubian and Nilotic goats showed 100% positive PCR products for primer K16. the frequency of distribution is similar for both. The number of nitrogen bases (1.0) is 200-700 bp.

PCR products of G12 primer:

Results given in table (5) indicated that the Nubian and Nilotic goats gave similar PCR products (95%) for primer G12. the frequency of distribution of this primer is 0.95 for each group (Fig 5). The number of nitrogen bases is 200-700 bp.

PCR products of Q3 primer:

Results given in table (6) showed that 47.5% of the local breeds goats gave PCR products for primer Q3. the Nilotic goats showed slightly higher (27.5%) PCR products compared the Nubian (20%). The frequency of the primer Q3 was 0.4 and 0.55 for the Nubain and Nilotic goats respectively (Fig.). Number of base pairs are 200-700 bp.

PCR products of K3 primer:

Data presented in table (7) indicated that (67.5%) of the Nubian and Nilotic goats gave PCR products for primer K3. the positive Nubain goats were slightly higher (35%) relative to the Nilotic ones. The frequency of distribution of the K3 primer is 0.7 and 0.65 in the Nubian and Nilotic goats respectively. Number of nitrogen bases are 200-900bp.

Table (3): Number and percentage of the Nubian and Nilotic goats that gave PCR product for Q19 primer.

sample	Q19		Total
	positive	negativeb	
Nubin goat	16 40.0%	4 10.0%	20 50.0%
Nilotic goat	18 45.0%	2 5.0%	20 50.0%
Total	34 85.0%	6 15.0%	40 100.0%

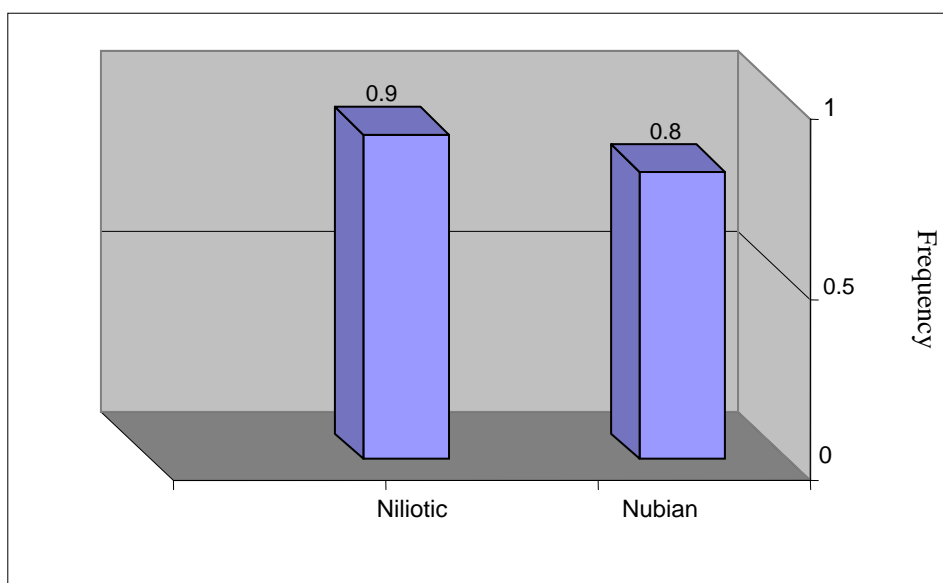


Fig (5): Frequency of primer Q19 in the Nubian and Nilotic goats.

Table (4): Number and percentage of the Nubian and nilotic goats that gave PCR product for K16 primer.

sample	K16	Total
	positive	
Nubin goat	20 50.0%	20 50.0%
Nilotic goat	20 50.0%	20 50.0%
Total	40 100.0%	40 100.0%

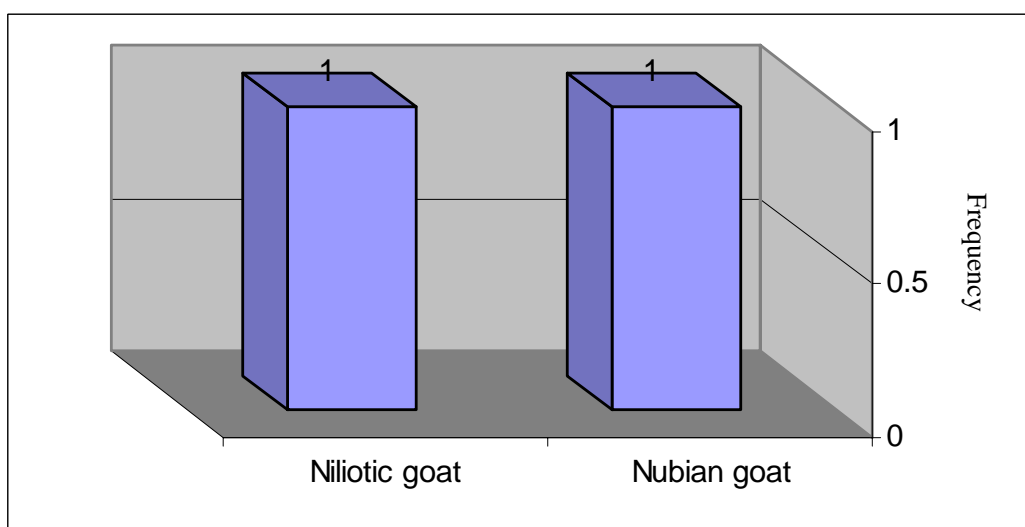


Fig (6): Frequency of K16 primer in the Nubian and Nilotic goats.

Table (5): Percentage of the Nubian and nilotic goads that PCR proudest for G12 primer.

sample	G12		Total
	positive	negativeb	
Nubin goat	19 47.5%	1 2.5%	20 50.0%
Nilotic goat	19 47.5%	1 2.5%	20 50.0%
Total	38 95.0%	2 5.0%	40 100.0%

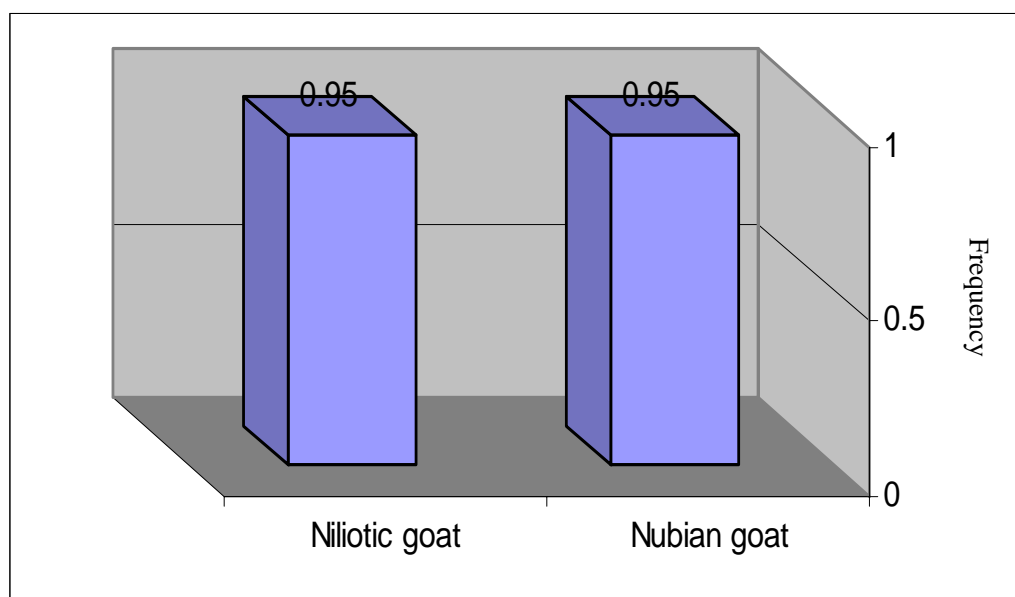


Fig (7): Frequency of G12 primer in the Nubian and Nilotic goats.

Table (6): shows number and percentage of the Nubian and nilotic goats that PCR proudest for Q3 primer

Table (6):.

sample	Q3		Total
	positive	negativeb	
Nubin goat	8 20.0%	12 30.0%	20 50.0%
Nilotic goat	11 27.5%	9 22.5%	20 50.0%
Total	19 47.5%	21 52.5%	40 100.0%

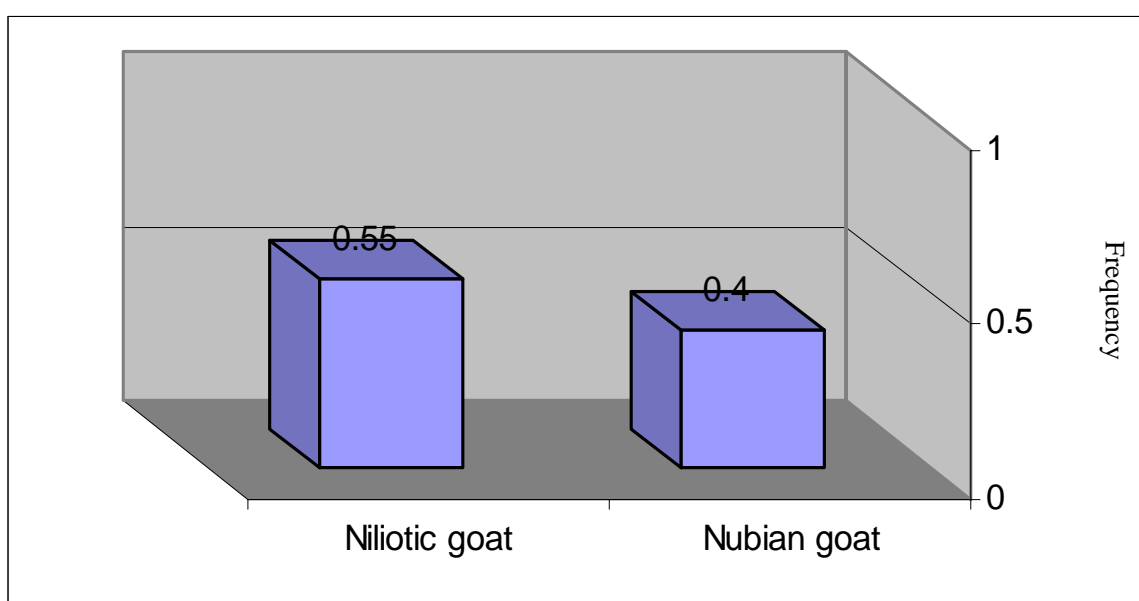


Fig (8): Frequency of Q3 primer in the Nubian and Nilotic goats.

Table (7): Number and percentage of the Nubian and nilotic goats that gave PCR product for K3 primer.

sample	K3		Total
	positive	negativeb	
Nubin goat	14 35.0%	6 15.0%	20 50.0%
Nilotic goat	13 32.5%	7 17.5%	20 50.0%
Total	27 67.5%	13 32.5%	40 100.0%

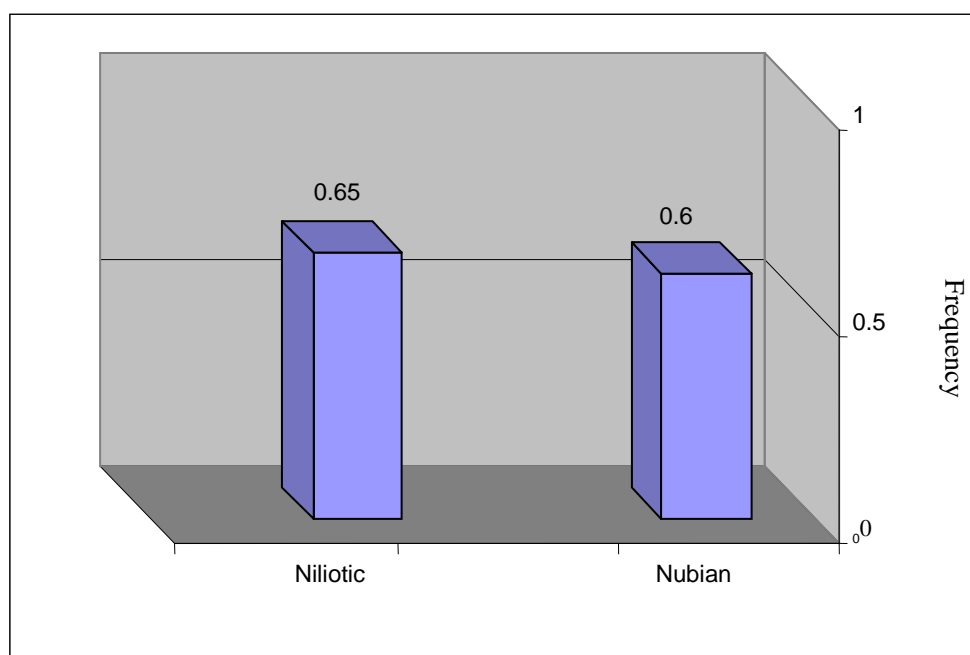


Fig (8): Frequency of K3 primer in the Nubian and Nilotic goats.

Chapter Four

Discussion

Results of this study indicated that the primers A11, A16 and Q7 showed no PCR product. Considering these primers as a genetic markers, it is evident that three genes were eliminated from our local breeds, without any apparent threat to their survival. The elimination of these genes may be due to the environment and / or our local breeds lack certain traits.

The PCR product of primer K16 table (4) of the Nubian and Nilotic goats (100%), reflects a great similarity among the foreign blood and our local breeds, this may be supported by the fragment length, which ranged between 200-700 base pairs. The results obtained for the primer G12 table (5) support the similarities between the Nubian and the Nilotic goats and reflect slight variability with respect to the foreign blood. The PCR products of Q19 table (3) indicated that the frequency of distribution of the gene decreased (0.825) relative to the foreign blood. Likewise the PCR product of the primer K3 table (7) indicate a further decrease in the frequency of the gene (0.675), whereas the difference between the Nubian and the Nilotic is negligible. On the other hand the PCR product of Q3 table (6) primer indicates greater difference between our local breeds and the foreign blood. (frequency 0.47), Although the number samples in this study is the small variation in the frequencies of the primer products, of the

local breeds are minor, thus the Nubian and Nilotic goats showed to some extent similar genotypic constitution with respect to the studied primers. The wide range of base pairs observed for all investigated primer are in agreement with results obtained for the same primer used for Chinese goat (LiLi et.al 2006).

Conclusion

This preliminary results indicated great similarities between the Nubian and Nilotic goats, and reflects some variability with the forgin goats.

Recommendation

Further studies are highly recommended which should include larger numbers and different types of goats to investigate the genetic diversity and possibility of improving local breeds through crossing with foreign blood.

References

- Aoad (1990)** Arab Organization Four Agricultural Development. Goat Resource's in Arab States -11- Sudan. (In Arabic). Aoad printing press, Khartoum Sudan.
- Arnau, J., A.P. Housego and R.P Oliver. 1999.** The use of RAPD markers in the genetic analysis of the plant pathogenic fungus *Cladosporium Fulvum*. *Curr. Genet.* 2 5:438-444.
- Borowsky R.L., Mccelland M., Cheng R., Welsh J. (1995).** Arbitrarily primed DNA Fingerprinting for polygenetic reconstruction in vertebrates: The Xiphophorus model. *Mol. Biol. Evol.*12 (6):1022-1032.
- Bowditch B.M.,Albright D.G.,Willams J.G.K.,Braun M.J(1993).** Use of randomly amplified polymorphic DNA markers in comparative genome studies. *Methods Enzymol.*224:294-309
- Bruford MW, Wayne RK (1993)** Microsatallites and their application to population genetic studies *Current Opinion in Genetics and Development* 3:939-943.
- Buchanan, F. C, Adams, L. J, Littlejohn, R. P., Maddox. JF .And Crawford, A. M, (1994).** Determination of evolutionary relationships among sheep breeds using Microsatellites. *Genomics.* 22:397-403.
- Bumstead, N & Playga J., (1992)** A preliminary Linkage Map of the Chicken Genome. *Genomic.* 13:690 - 697.
- Callen DF, Thompson AD, Sheen Y, Phillips HA, Richards RI, Mulley JC, Sutherland GR (1993)** Incidence and origin of Null alleles

in the (AC) n microstallite markers. American Journal of Human Genetic 52:922-27.

Cargill, S.L., G.B. Anderson and J.F Medrano. (1995). Development of a species-specific marker using RAPD analysis to distinguish between sheep and goats. Anim.Biotechnology .6:93-100.

Clark A.G., Lanigan C.M.S. (1993). Prospects for estimating nucleotide divergence with RAPDs Mol. Biol. Evol. 10 (5): 1096-1111.

Cregon PB, Bhagwat AA, Akkaya MS, Rongwen J (1995). Microstallite fingerprinting and mapping in soybean. Methods in Molecular Cell Biology 5:49-61.

Cushwa W, Dodds k, Crawford A, Medrano J (1996) Identification and genetic mapping of random amplified polymorphic DNA (RAPD) markers to the sheep genome Mammalin genome 7.580-58.

Dodgson, J.B., Cheng, H.H& Okimoto, R., 1997. DNA marker technology: A revolution in animal genetics. Poultry Sci. 76:1108-1114.

Elnaim, Y.A. (1979). Some productive traits of Sudan Nubian goats. M.V.SC Thesis University of Khartoum.

French, M.H (1970). Observation on goat's. FAO. Agricultural studies No, 80, Rome, Italy.

Goldstein, D. B & Pollock, D. D., 1997. Launching microstalleties : A review of mutation process and methods of polygenetic inference. Journal of Heredity 88:335-342.

Groenen, M.A.M., Cheng. H., Bumsteat ,N., Benkel, B.F., Briles, W.E., Burket, T., Burt, D.W., Crtitenden, L.B., Dodgson ,J., Hillel

- ,J., Lamont, S., Poncedeleon, A. Soller, M., Takashi, H.& Vignal, A., (2000).** A consensus map of the chicken genome. *Genome Research*. 137-147 pp.
- Haberfeld,A.,Cahaner,A.,Yoffe,O.,Plotsky, Y, and Hillel, J.(1991)** *Anim. Genet* 22:299-305.
- Hadrys, H., M. Batik and B. Schierwater. (1992).** Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol.Ecol.*1:55-63.
- Hayes, H., Elduque, M., Gautier, L. Schiblere., Cribiu, E.& Eggen, A., (2002).** Gene mapping progress in cattle and updated comparative map with man, mouse, rat and pig. *Proc. xxviii International conference on Animal Genetics*, august 11-15, 2002 Gottingen Germany.
- Herbergs, J., Siweck, M., Crooigmans, R.P.M.A., Vanderpoel, J.J & Groenen, M.A.M., (1999).** Multicolor fluorescent detection and mapping of AFLP markers in Chicken (*Gallus domestic us*). *Anim. Gen.* 30, 274-285.
- Jarne P, Lagoda PJJ (1996)** Microsatellites, from molecules to populations and back. *Trends in Ecology and evolution* 11:424- 429.
- Kantanen J, Vikki J, Elo K, Maki-Tanila A (1995)** Random amplified polymorphic DNA in cattle and sheep: application for detecting genetic variation. *Anim Genet* 26: 315-320.
- Karp a, Kresovich S, Bhat KV, Ayand WG, Hodgkin (1997)** Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI Technical Bulletin No. 2, International Plant Genetic Resources Institute, Rome, Italy. Available at <http://198.93.227.125/publicat/techbull/TB2.pdf>.

- Kiwuwa, G.H (1986).** Breeding strategies for small ruminant production in eastern and South Africa.1. Breed characters and general performance. Proceeding of workshop on improvement of small ruminants in eastern and South Africa. Nairobi, Kenya, 16-22 October 1986.
- Krauss SL (1999)** complete exclusion of nonsires in an analysis of paternity in a natural plant population using amplified fragment length polymorphism (AFLP). *Molecular Ecology* 8:217-226.
- Kruger, K, Stranizinger, G. & Rider, S.,(2002).** A full genome can panel of horse (*Equus caballus*) microsatellite markers applied to different equid species. Proc. xxviii International conference on Animal Genetics, august 11-15, 2002 Gottingen, Germany.
- Larrsa J, Garscia A, Ambrose NC, Alonso JM, Para A, de Mendoza MH (2002)** Simple random amplified polymorphic DNA genotyping method for field isolates of *Deratophilus congolensis*. *J Vet Med B infect Dis Vet Public Health*.
- Lee, J.C.1. And J, - G. Chang. (1994).** Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species identification. *Forensic Sci. Internal*. 67:103-107.
- LiLi, Jie Zhang, Jing, que Zhus, Song Gu, Oun Sun, Guang-ming Zhou, Chang-xiu, Qiang Li, Lin-yu chen, Da-xu Li, Suryun Liu and Zhi-rang, Yang (2006)** *J. Zoo. Logical science* 23;229-234, Japan.
- Machugh, D.E., Shriver, M.D, Loftus, R.T., Cunningham, P. & Bradely, D.G., (1997).** Micro satellite DNA variation and the evolution, domestication and phylogeography of Taurine and Zebu cattle (*Bos Taurus* and *Bos indicus*). *Genetics*. 146: 1071- 1086.

- Mackenzie, D. (editor). (1957). Goat husbandry. Third edition (1970).**
Faber and Faber ltd, London, U.K.
- Mason, I. (Editor). (1951) World Dictionary of live stock breeds and varieties. CAB international third edition (1988). Edinburgh Scotland.**
- Matthes MC, Daly A, Edwards KJ (1998)** Amplified fragment length polymorphism (AFLP). In: Karp A, Isaac PG, Ingram DS (Eds.). Molecular Tools for Screening Biodiversity. Chapman and Hall, London, pp. 183-190.
- Mollenbeck M (1999)** Genetic relationship of 32 cell lines of the *Explodes octocarinatus* species complex revealed by random amplified polymorphic DNA (RAPD) fingerprinting. Mol Ecol 8:1971-1979.
- Morand-Fehr (2004)** Proposals for improving the research efficiency in goats. Small Ruminant Res 51: 145-153.
- Morgante M, Piffer A, Jurman I, Puglia G, Olivieri (1998)** Isolation of microsatellite markers in plants. In: Karp A, Isaac PG, Ingram DS (Eds.). Molecular Tools for Screening Biodiversity. Chapman and Hall, London, pp.288-296.
- Moxon ER Wills C (1999)** DNA microsatellites: agents of evolution/ Scientific American, January: 72-77.
- Muffarch, M.B. (1995).** Goats' breeds and varieties in Sudan. Proceedings of training course on sheep and goat production, held by Arab Center for studies of Arid zones and dry land (ACSAD), Khartoum, 17-27 January, 1995.
- Mullis, K.B. and Faloona, F.A. (1987).** Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. 155: 335-350.

- Nakajima Y, Oeda K, Yamamoto T (1998)** Charactersation of genetic diversity of nuclear and mitochondrial genomes in *Daucus* vareties by RAPD and AFLP. *Plant Cell Reports* 17:848-853.
- Nicholas. F.W., (1996).** Introduction to veterinary genetics. Oxford University Press.
- Nijman I.J., Bradley, D.G., Hanotte, O., Osten, M. & lenstra, J.A., (1999).** Satellite DNA polymorphisms and AFLP correlate with Bos–indicus–Taurus hybridization. *Anim. Gen.* 30, 256-273.
- O`brien, S.J, (1991).** Mammalian genome mapping: Lessons and prospects, *Current Opinion in Genetics and Development.* 1:105-111.
- Qian W, Ge S (2001).** Analyses of population hereditary constitution by usingdominates markers. *Act Genet Sini* 28: 244-255 (in Chinese).
- Rohrer, G. A AND Keele, J.W., (1998a).** Identification of quantitative trait loci affecting carcass composition in Swine: I. Fat deposition traits. *J. Anim. Sci.*76:2247-2254.
- Rohrer, G.A AND Keele, J.W., (1998b).** Identification of quantitative trait loci affecting carcass composition in Swine: ii. Muscling and Wholesale product yield traits. *J. Anim. Sci.*76:2255-2262.
- Schiumenti, J. (1998).** Global analysis of gene function in mammals: integration of physical, mutational and expressionstrategies. *EJB Electronic Journal of Biotechnology.*
- Singh RV, Sharma D (2002)** within-and between strain genetic variability in White Lenghorn population detected through RAPD markers. *Br Poult Sc* 43:33-37.
- Smith, E.J, Jones, C.P., Bartlett, J. & Nestor, K.E., (1996).** Use of randomly amplified polymorphic DNA markers for the genetic

analysis of relatedness and diversity in chickens and turkeys.
Poultry Sci.75:579-584.

Spike, C.A., Bumstead, N., Crittenden, L.B. & Lamont, S.J., (1996).
PFRL mapping of expressed sequence tags in the chicken. Journal
of Heredity 87:6-9.

Strand M, Prolla TA, Liskay RM, Petes TD (1993) Destabilization of tract
of simple repetitive DNA in yeast by mutations affecting DNA
mismatch repair. Nature 365:274-276.

Tautz, D., & Renz, M., (1984). Simple sequences are ubiquitous
repetitive components of eukaryotic genomes .Nucleic Acids
Research 17:16-

Tautz, D., (1989). Hypervariability of simple sequences as a general source
for polymorphic DNA markers. Nucleic Acids Research 17:16.

**Vanhala, T., Tuiskul-Haavisto, M., ELO, K., Vilkki, J., & Maki-Tanila,
A., (1998).** Evaluation of genetic variability and genetic distances
between eight chicken lines using microsatellite markers. Poultry
Sci.77:783-790.

Vanzevern, A., Peelman, L., Vandeweghe, A. & Bouquet, Y., (1995). A
genetic study of four Belgian pig populations by means of seven
microsatellites loci. J. Animals Breeding & Genetics .112:191:204.

**Von, P, Hogers, Pene, Bleeker, M., Reijans, M., Vandele, T., Hornes,
Miranda, Frijters, Adrie, Pot, Jerina, Peleman, J., Kupier, M.
& Zabeau. M., (1995).** AFLP: A new technique for DNA
fingerprinting .Nucl. Acids Res. 23(21):4407-4414.

Walsh, B., (2002). Minireview: Quantitative Genetics in the Age of
Genomics. Theoretical populations Biology 59, 175-184.

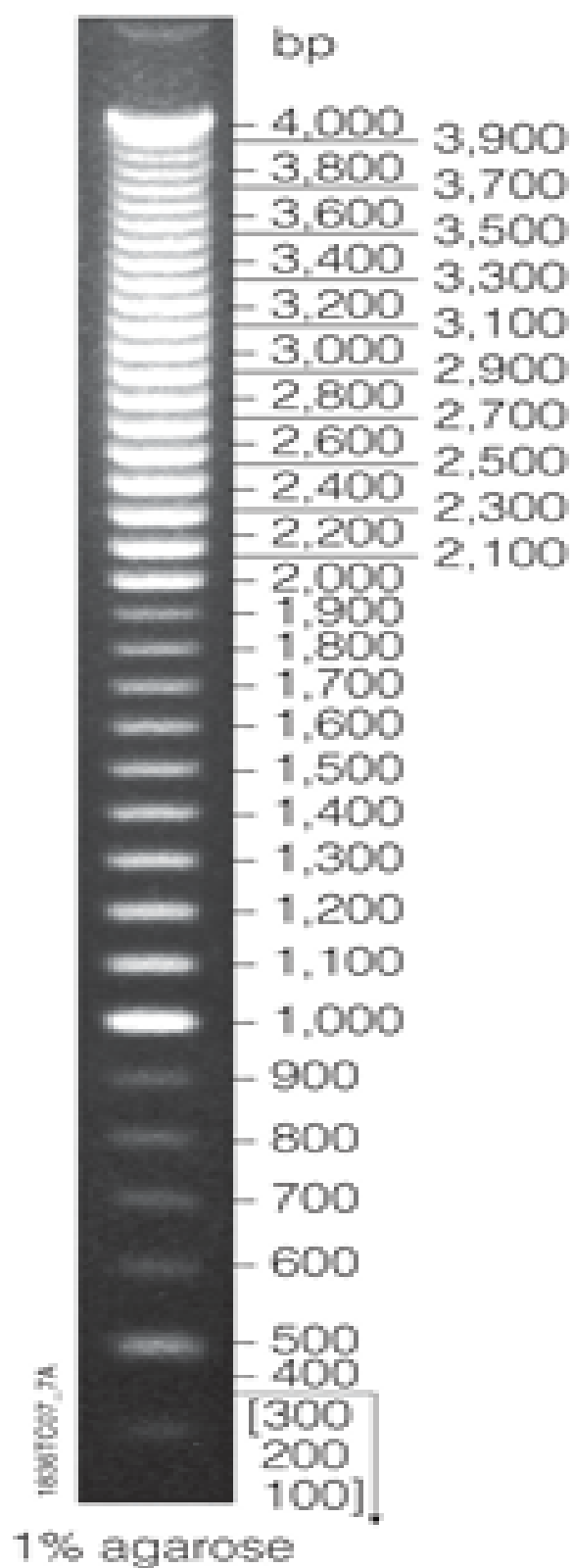
- Wang, L., YU, T.-P., Tuggle. C.K., LIU, H.-C. & Rothschild D, M.F., (1998).** A directed search for quantitative trait loci on chromosome 4 and 7 in pigs. *J. Anim. Sci.* 76:2560-2567.
- Welsh, J., Mcclelland M. (1990).** Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* Vol. 18:No.24.
- Welsh, J., McClelland, M. (1990)** *Nucl. Acids Res.* 18:7213-7218.
- Willms, J, GK. Kubellk A.R., Livak K.J. Rafalski J.A., Tingey S.V. (1990).** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* Vol.18:No.22.
- Yu Y, Lain L, When J, Shi X, Zhu F, Nie L (2004).** Genetic diversity and relationship of Yunnan native cattle breeds and introduced beef cattle breeds. *Biochem Genet* 42:1-9 (in Chinese).



Appendix (1): Nubian goat



Appendix (2): Nilotic goat



Appendix (3) : Ladder, molecular size of DNA marker (100 bp).